Vol. XLVI January, 1924 No. 1

BIOLOGICAL BULLETIN

STUDIES ON THE INFLUENCE OF INANITION ON THE DEVELOPMENT AND THE DURATION OF LIFE IN INSECTS.

STEFAN KOPEĆ,

Government Institute for Agricultural Research, Pulawy, Poland.

With 3 Tables.

CONTENTS.

The experiments were performed on the caterpillars of the common moth *Lymantria dispar* L. Each experiment consisted of several separate broods. The caterpillars deriving from one brood were divided in two parts and put in two jars, one part was subjected to starvation, the other, daily fed, was used as a control. The inanition applied in these studies was total but intermittent, the caterpillars being totally deprived of food during certain days and fed *ad libitum* during the remaining. The distribution of these days was different in different series of experiments, as may easily be seen from tables 1 to 3. The obtained pupæ being kept each separately, it was possible to study the influence of starvation not only on the duration of the stage of caterpillars, but also on that of each chrysalis and each moth.

In the chief experiments (series *A* to *F*) the whole material consisted of 1239 starved caterpillars, and 843 specimens for control; 547 pupæ were obtained from the caterpillars subjected to starvation and 763 from control larvæ; they produced 476 "starved" and 683 control moths (Cf. Table I.). The increased

[1] Paper from the Laboratory of Experimental Morphology., cf. *Mém. de l'Institut Nat. Polonais d'Économie Rurale à Pulawy*, Vol. 1, 1921.

TABLE I

Onset of Experiments.	Starvation Applied to:	Number Referring to Each Brood.	Number of Pupae. Starved. ♂♂	Number of Pupae. Starved. ♀♀	Number of Pupae. Control. ♂♂	Number of Pupae. Control. ♀♀	Number of Moths. Starved. ♂♂	Number of Moths. Starved. ♀♀	Number of Moths. Control. ♂♂	Number of Moths. Control. ♀♀	Weight of Pupae in Mg. ♂♂. Starved. Limits of Individual Fluctuations.	♂♂. Starved. Average.	♂♂. Control. Limits of Individual Fluctuations.	♂♂. Control. Average.	♀♀. Starved. Limits of Individual Fluctuations.	♀♀. Starved. Average.	♀♀. Control. Limits of Individual. Fluctuations.	♀♀. Control. Average.
Since hatching from eggs.	*Series A.* Starvation lasting one day every second day (+ − + − + −) applied during the whole larval life.	1	10	14	21	35	7	11	18	28	180–394	252.7	262–524	383.7	433–935	686.2	697–1277	1006.3
		3	9	12	16	15	9	11	15	15	302–421	364.0	410–565	507.1	629–1158	963.0	872–1570	1261.6
		4	15	16	15	30	12	14	12	24	215–480	304.6	180–471	376.8	612–1181	907.1	420–1264	870.6
		11	10	13	35	38	10	12	30	31	225–317	271.3	184–572	430.5	348–852	698.5	485–1605	1057.4
	Series B. The same starvation applied only during the first 20 days of larval life.	2	10	14	14	24	8	11	12	22	222–433	345.3	271–471	394.4	755–1382	1087.7	611–1535	1175.0
		9	12	8	15	16	12	8	12	11	395–565	439.6	280–556	388.0	470–1557	1097.2	538–1354	1030.8
		12	10	10	17	16	8	10	14	10	291–502	416.2	275–514	399.3	563–1504	1148.8	598–1521	1073.1
Since the last moult but one.	*Series C.* Starvation lasting one day every third day (+ + − + + −)	34	15	9	12	9	14	8	12	9	340–425	366.8	468–533	496.1	760–1015	876.0	1257–1465	1341.6
		35	18	11	18	8	14	10	18	7	273–395	333.7	447–586	509.1	414–793	577.8	624–1587	1273.5
		36	16	10	16	12	10	10	14	12	232–355	314.2	344–511	444.2	322–1280	833.6	996–1081	1034.5
		37	12	12	16	10	12	12	14	10	247–372	303.3	335–507	409.0	620–917	814.2	820–1230	1042.6
		38	15	18	16	16	12	14	16	16	255–369	306.6	419–557	466.2	444–1063	733.6	850–1284	1074.8
		39	9	9	11	15	7	8	9	12	200–396	291.7	373–537	447.7	375–739	581.1	734–1576	1131.8
	Series D. Starvation lasting one day every second day (+ − + − + −)	28	11	8	13	9	9	8	12	8	163–274	212.2	298–454	399.1	530–782	665.8	962–1293	1146.3
		29	13	13	15	15	13	12	15	12	171–212	191.5	431–490	452.4	363–590	475.0	745–1320	1115.0
		30	16	9	15	10	16	7	15	10	185–302	242.5	401–567	445.4	290–638	469.3	867–1285	1052.4
		31	12	12	14	14	12	10	13	14	185–279	232.0	409–530	477.4	382–963	595.7	1147–1565	1329.4
		32	12	12	12	13	12	9	12	12	145–298	230.1	279–500	419.5	373–1085	689.6	663–1352	1176.9
		33	12	10	10	10	9	8	9	9	155–195	168.6	343–537	439.1	347–695	489.4	808–1350	1150.3
	Series E. Starvation lasting two days every second day (+ − − + − −)	40	6	8	7	14	6	6	6	14	121–207	154.0	422–552	480.2	156–283	230.8	715–1371	1138.0
		42	4	6	10	11	3	6	10	10	118–168	135.6	391–427	411.6	131–378	269.1	705–1302	1059.3
		45	6	6	13	10	6	4	13	10	100–131	112.5	355–540	446.8	172–421	318.2	844–1355	1088.3
	Series F. Starvation lasting two days every third day (+ + − − + + − −)	46	10	8	14	16	6	7	14	14	165–272	173.7	365–525	443.4	299–590	428.1	1112–1860	1349.5
		47	8	10	8	16	8	10	6	14	158–210	182.5	445–602	516.2	355–486	418.0	922–1528	1130.7
		48	8	10	14	14	7	8	14	14	155–257	172.8	330–556	505.7	289–473	395.2	913–1052	998.6

mortality of the starved caterpillars ought not to be ascribed first of all to the emaciating influence of inanition, the chief cause of this mortality is the moult. Directly after moulting the animal takes very much food, as during these processes it does not eat at all and only digests its own substances. Therefore when the fasting day falls on the period succeeding moult the caterpillars are often unable to resist starvation. The mortality of chrysalids, on the contrary, was identical in starved and in control specimens. The willow leaves on which the caterpillars were fed were always of the same variety and of the same freshness. The conditions of space, light and moisture were identical in all jars during the whole t me of observations and the temperature was from 16° to 19° C. Food was put in great superfluity into control jars every morning and into those containing starved material during the feeding days and it preserved its freshness the whole day. All experiments were performed during one season (1920).

1. The Influence of Inanition on the Duration of Separate Developmental Stages.

For the experiments of series *A* caterpillars belonging to four different broods were used, from 1 to 6 hours after hatching. These caterpillars were deprived of food every second day, in the remaining days they were fed *ad libitum*. Starvation lasting one day and applied every second day may be designated by the symbol + − + − + −. Table II. records the limits of individual fluctuations in the duration of life of the caterpillars and chrysalids belonging to each brood, as well as the average duration of life in starved and in control specimens of the two sexes. Table III. finally shows the average differences of the duration of larval and of pupal life of individuals deprived of food for each brood separately, calculated in percentages of the average duration of the larval or of the pupal stages of control specimens of the same brood and the same sex. From these tables we see that the life of caterpillars subjected to such starvation was considerably prolonged. In all lots without exception the longest-lived control caterpillars underwent pupation earlier than the shortest-lived starved specimens of the same brood and sex. (Cf. Table II. *Series A*). If we take the average of the averages of all broods of series *A*, a quantity which I shall call "average of broods,"

Table II

Onset of Experiments.	Starvation Applied to:	Number Referring to Each Brood.	Larval Life, ♂♂, Starved: Limits of Individual Fluctuations.	Larval Life, ♂♂, Starved: Average.	Larval Life, ♂♂, Control: Limits of Individual Fluctuations.	Larval Life, ♂♂, Control: Average.	Larval Life, ♀♀, Starved: Limits of Individual Fluctuations.	Larval Life, ♀♀, Starved: Average.	Larval Life, ♀♀, Control: Limits of Individual Fluctuations.	Larval Life, ♀♀, Control: Average.	Pupal Life, ♂♂, Starved: Limits of Individual Fluctuations.	Pupal Life, ♂♂, Starved: Average.	Pupal Life, ♂♂, Control: Limits of Individual Fluctuations.	Pupal Life, ♂♂, Control: Average.	Pupal Life, ♀♀, Starved: Limits of Individual Fluctuations.	Pupal Life, ♀♀, Starved: Average.	Pupal Life, ♀♀, Control: Limits of Individual Fluctuations.	Pupal Life, ♀♀, Control: Average.
Since hatching from eggs.	*Series A.* Starvation lasting one day every second day (+ – + – + –) applied to during the whole larval life.	1	62–80	70.7	42–49	43.8	75–81	78.0	43–58	46.0	11–16	14.7	19–23	22.2	10–12	11.0	17–21	19.8
		3	63–74	67.6	42–47	45.0	75–81	78.5	45–55	48.0	15–18	16.6	22–25	23.4	10–12	11.0	20–22	20.8
		4	63–84	71.8	44–49	45.7	71–81	76.5	44–68	49.2	12–20	16.0	22–25	23.8	10–13	11.2	17–21	20.2
		11	62–67	64.5	43–49	45.3	75–78	76.0	44–59	48.2	14–19	16.5	21–26	23.2	11–12	11.6	19–22	20.0
	Series B. The same starvation applied only during the first 20 days of larval life.	2	56–70	61.1	43–55	45.8	60–67	63.0	44–59	48.6	16–21	19.2	21–25	23.7	15–17	16.0	18–22	20.4
		9	57–69	62.5	44–68	49.4	63–72	68.3	45–68	52.5	17–21	19.0	18–24	23.0	13–17	15.3	16–21	20.1
		12	63–77	69.3	44–69	54.4	64–71	67.6	44–64	51.6	14–20	17.8	16–24	21.2	14–18	16.0	18–22	20.6
Since the last moult but one.	*Series C.* Starvation lasting one day every third day (+ + – + + –)	34	21–23	22.2	18–20	18.8	23–37	28.1	19–20	19.6	22–23	22.2	22–24	23.1	16–23	19.8	20–22	21.0
		35	21–24	22.4	18–20	18.8	26–36	30.0	20–27	21.5	21–24	22.5	22–24	23.2	16–23	19.8	20–22	21.0
		36	20–28	22.5	18–21	19.6	24–36	29.4	20–22	20.6	21–22	21.6	21–23	22.2	16–21	19.0	19–22	20.1
		37	21–28	23.3	19–21	19.7	23–31	26.2	20–21	20.4	20–23	21.5	20–25	22.4	18–20	19.2	20–21	20.6
		38	20–22	21.4	18–22	19.5	22–28	23.8	20–22	20.6	22–24	23.2	23–25	24.0	19–20	19.7	20–22	20.7
		39	21–32	23.3	19–20	19.8	22–35	26.3	19–26	21.1	21–23	22.1	21–24	22.8	18–20	19.3	19–21	20.2
	Series D. Starvation lasting one day every second day (+ – + – + –)	28	24–30	27.0	16–22	18.0	26–29	26.8	17–21	19.2	20–22	21.3	22–23	22.6	17–22	19.8	20–22	21.0
		29	25–27	26.0	16–19	17.8	26–30	28.0	18–22	19.4	21–23	22.0	22–24	22.8	18–20	19.3	20–21	20.7
		30	23–25	24.0	17–19	18.0	23–39	29.5	16–20	18.2	21–22	21.5	23–25	23.8	16–20	18.4	20–21	20.6
		31	24–25	24.5	17–20	18.5	23–39	28.8	18–23	20.5	21–23	22.0	21–24	23.0	19–22	20.5	21–23	21.7
		32	23–33	26.3	17–22	19.1	23–38	30.2	18–25	20.1	20–24	22.0	21–24	22.9	16–21	19.0	19–22	20.3
		33	24–33	29.0	18–22	20.1	27–43	35.1	20–23	21.2	21–22	21.6	22–24	23.2	14–20	17.3	20–23	21.0
	Series E. Starvation lasting two days every second day (+ – – + – –)	40	27–32	29.3	18–19	18.4	30–46	37.0	18–23	19.5	16–21	18.6	22–24	22.8	13–15	14.3	20–22	21.0
		42	25–38	31.2	18–20	18.8	28–45	37.0	18–22	19.3	17–19	18.0	22–23	22.4	12–15	13.6	19–21	19.7
		45	26–35	31.3	17–21	18.3	28–48	37.1	19–21	19.8	16–19	17.3	22–25	23.8	13–16	14.7	19–21	20.3
	Series F. Starvation lasting two days every third day (+ + – – + + – –)	46	22–38	28.9	18–20	18.7	33–39	36.5	18–24	19.8	17–21	19.8	21–23	22.4	14–16	15.1	19–22	20.2
		47	32–34	33.0	19–24	20.8	37–39	38.0	20–24	21.7	18–20	19.0	23–24	23.3	13–16	14.5	20–21	20.4
		48	21–39	27.1	18–19	18.1	29–37	33.2	18–20	19.0	17–21	19.4	23–25	23.8	15–18	16.5	20–21	20.6

Table III

		Experiment Begun Since Hatching from Eggs.									Experiment Begun Since the Last Moult But One.																					
		Series A Starvation Lasting One Day Every Second Day (+ − + − + −) Applied During the Whole Larval life.					*Series B.* The Same Starvation Applied to Only During the First 20 Days of Larval Life.				*Series C.* Starvation Lasting One Day Every Third Day (+ + − + + −).							*Series D.* Starvation Lasting One Day Every Second Day (+ − + − + −).							*Series E.* Starvation Lasting Two Days Every Second Day (+ − − + − −).				*Series F.* Starvation Lasting Two Days Every Third Day (+ + − − + + − −).			
		Number Referring to Each Brood.				Average of Broods.	Number Referring to Each Brood.			Average of Broods.	Number Referring to Each Brood.						Average of Broods.	Number Referring to Each Brood.						Average of Broods.	Number Referring to Each Brood.			Average of Broods.	Number Referring to Each Brood.			Average of Broods.
		1	3	4	11		2	9	12		34	35	36	37	38	39		28	29	30	31	32	33		40	42	45		46	47	48	
Average prolongation of life of the starved caterpillars in per cent. of the average duration of larval life of control specimens of the same brood and sex, from the time of onset of experiment till pupation.	♀♀	61.4	50.2	57.1	42.3	*52.7*	33.4	26.5	27.3	*29.0*	18.0	19.1	14.7	18.2	9.7	17.6	*16.2*	50.0	46.0	33.3	32.4	37.6	44.2	*40.5*	50.2	65.9	71.0	*65.3*	54.5	58.6	49.7	*54.2*
	♂♂	69.5	63.5	55.4	57.6	*61.5*	29.2	30.0	31.0	*30.0*	43.3	39.5	42.7	28.4	15.5	24.6	*32.3*	39.5	44.3	62.0	40.4	50.2	65.5	*50.3*	89.7	91.7	87.3	*89.5*	84.3	75.1	74.7	*78.0*
Average abbreviation of life of the "starved" pupæ, in per cent. of the average duration of pupal life in control specimens of the same brood and sex.	♀♀	33.7	29.0	32.7	28.8	*31.0*	18.9	17.3	16.0	*17.4*	3.8	3.0	2.7	4.0	3.3	3.0	*3.3*	5.7	3.5	9.6	4.3	3.0	6.8	*5.6*	18.4	19.6	27.3	*21.7*	11.6	18.4	18.4	*16.1*
	♂♂	44.4	47.1	44.5	42.0	*44.5*	21.5	23.8	22.3	*22.5*	5.7	5.7	5.0	6.7	4.8	4.4	*5.3*	5.7	6.7	10.6	5.5	6.4	17.6	*8.7*	31.9	30.9	27.5	*30.1*	25.2	28.8	19.9	*24.6*
Average weight of the "starved" pupæ in per cent. of the average weight of control chrysalids of the same brood and sex.	♀♀	65.5	71.7	80.9	63.0	*70.2*	87.5	113.2	104.2	*101.6*	73.9	65.5	70.7	74.1	65.7	65.1	*69.1*	53.1	42.3	54.4	48.5	54.8	38.3	*48.5*	32.0	32.9	25.1	*30.0*	39.1	35.3	34.1	*36.1*
	♂♂	68.1	76.3	104.1	66.0	*78.3*	92.5	106.4	107.0	*101.9*	65.2	45.3	80.5	78.0	68.2	51.3	*64.7*	58.0	42.6	44.5	44.8	58.5	42.5	*48.4*	20.2	25.4	29.2	*24.9*	31.7	36.9	39.5	*36.0*

we obtain for male specimens a prolongation of larval life of 52.7 per cent. for the female of 61.5 per cent. Cf. Table III. As the processes of pupation were checked every 24 hours and the caterpillars were taken for the experiments from 1 to 6 hours after their hatching from eggs, the error concerning duration of the larval stage could have in no case exceeded 30 hours.

The duration of the pupal stage is influenced by the starvation of caterpillars in an essentially different manner, viz, the chrysalids which have developed from starved caterpillars undergo transformation into adult moths far earlier than control pupæ, but the abbreviation of the pupal stages is smaller than the prolongation of larval life. This abbreviation amounted in the "averages of broods" in males to 31.0 per cent. in females to 44.5 per cent. of the average duration of pupal stage of control specimens. Pupation having been controlled every 24 hours, emergence of moths every 12 hours, the error in estimation of the duration of the pupal stage could not exceed 36 hours.

The moths of *Lymantria dispar* L. do not take any food in their imaginal stage. Control males lived as moths in separate lots from 1 to 8, from 5 to 8, from 2 to 8 and from 1 to 8 days and the "starved" specimens from 4 to 5, from 5 to 11, from 2 to 8 and from 1 to 6 days. Normal females lived from 3 to 13, from 1 to 10, from 1 to 12 and from 2 to 14 days, those derived from starved caterpillars from 5 to 6, from 3 to 10, from 7 to 10 and from 6 to 10 days. Comparing the mean values of the duration of life of the normal and of the "starved" moths we obtain in most cases a certain prolongation of life of the moths derived from starved caterpillars (+) though rarely a certain abbreviation (−). This prolongation or abbreviation calculated in days amounts in separate broods in the males to + 0.3, + 2.5, + 0.1 and −0.1 days, in the females to −2.4, + 0.3, + 2.8 and + 2.1 days. We see that starvation of caterpillars has no distinct effect on the duration of the imaginal stage. Emergence and death of moths having been checked every 12 hours, the error in the estimation may attain only 24 hours. The moths of the two sexes experimented upon both control and "starved" have never been allowed to mate.

If we take into consideration the behavior of the "starved" moths as well as the fact that the abbreviation of the pupal period was smaller than the prolongation of the larval stage, we must draw the conclusion that total deprivation of food of caterpillars every second day has a considerable positive effect on the total duration of their life from hatching until death. The prolongation of the whole developmental period amounts here in the average of broods in males to 16.5 and in females to 20.4 days, or in percentages relative to the duration of life of control specimens in males to 24.2 per cent. and in females to 30.0 per cent.

The above results find complete confirmation in my farther comparative experiments in which inanition of various intensity was applied (series *C*, *D* and *E*). In these experiments caterpillars were used from 1 to 12 hours after their last moult but one. In series *C* food was administered two days, the third day the insects were starved (starvation lasting one day every third day + + − + + − + + −). In series *D* the animals were again deprived of food every second day (starvation lasting one day every second day + − + − + −) and the caterpillars of series *E* were deprived of food two days and ate only every third day (starvation lasting two days every third day + − − + − − + − −). From the absolute figures of Table II. as well as from the percentages in Table III we may infer that the duration of the larval period undergoes also, in these starved animals considerable prolongation (cf. analogous results obtained in silk-worms by Kellogg and Bell, '04 *b*), simultaneously with abbreviation of the pupal stage. These changes become more and more remarkable in proportion as inanition increases, they are larger in series *D* than in series *C* and the largest in series *E* in which the fasting days were the most numerous. Also in these comparative experiments the prolongation of the larval life was greater than the abbreviation of the pupal period, while the duration of the imaginal life remained unchanged. Hence it follows that the total duration of the life of the insects from the beginning of the experiment till death of the moth, is more and more increased in proportion as inanition increases within the limits of the experiments. The duration of development from the last moult but one till the emergence of the moth underwent in the average of broods a prolongation (calculated in percentages of the average duration of such control periods) which in the males amounted to 5.6 per cent. in series *C*, to 15.1 per cent. in series *D* and to 16.8 per cent. in series *E*; in the females the prolongation was 13.3 per cent. in series *C*, 20.2 per cent. in series *D* and 28.6 per cent. in series *E*.

In series *F* the caterpillars were fed during two days and deprived of food during the next two. (Starvation lasting two days every third day ++−−++−−). Notwithstanding a different distribution of the feeding and fasting days, the quantitative relation of these days was the same as in series *D* to which starvation lasting one day every second day was applied (+−+−+−); in both experiments the caterpillars experimented upon were of the same age. It nevertheless turned out that the prolongation of life was much greater in caterpillars of series *F* than in specimens belonging to series *D*. (Cf. Table II. and III.) In series *F* it amounted in the average of broods in the males to 54.2 per cent. of the average of the life of control caterpillars from their last moult but one, and in the females to 78.0 per cent while in series *D* the corresponding numbers were 40.5 per cent. in males and 50.3 per cent. in females. The abbreviation of the pupal period calculated similarly was in series *F*, in males 16.1 per cent of the duration of life of control chrysalids and in females 24.6 per cent. while in series *D* the life of male pupæ underwent abbreviation of 5.6 per cent. and of the female 8.7 per cent. We see that changes of the duration of the larval and of the pupal stages induced by inanition depend not only on the mutual quantitative relation of the fasting and feeding period, but also on the distribution of these periods. The organism responds by more energetic reaction to longer, though less frequent, fasting intervals than to more frequent but shorter periods of starvation.

3. Inanition and its Bearing on the Physiology of Insect Metamorphosis.

When we try to discuss the foregoing results on the basis of hitherto existing references in the literature, we meet certain discrepancies which, however, may be cleared up by consideration of the physiology of animal metamorphosis.

Kellner ('87) fed caterpillars of the silk-worm during their whole life on insufficient quantities of leaves, and obtained a short prolongation of the larval stage. Analogous experiments were afterwards made by Kellogg and Bell ('04 *a* and *b*). Pictet ('05) came to the conviction that the larval stage is prolonged and the pupal stage shortened by feeding caterpillars on plants containing few food-stuffs, but that the conjoint periods of development do not undergo any changes. A prolongation of larval life caused by inadequate food was lately determined by Northrop ('17) Tangl. ('09*a*), finally, maintains that the larvæ of flies reared on pure egg-white which they refused to take underwent transformation approximately a week later than normally. These results are, in general, concordant with my observations on starved caterpillars. On the other hand, the observations of Krizenecky ('14) and of Szwajsówna ('16) on the larvæ of *Tenebrio molitor* which underwent metamorphosis earlier when totally starved contradict the above determinations. A similar discrepancy may also be remarked in analogous investigations on amphibians. While Barfurth (87) and others have ascertained that starvation of amphibians causes acceleration of their metamorphosis, other investigators induced retardation of these processes by the administration of poor food to tadpoles. It has been emphasized by Wolterstorff ('96), Morgan ('07), Kaufman ('18) and others that in amphibians the influence probably differs in different developmental stages of the tadpoles used for starvation. The former of the above mentioned investigators deprived large tadpoles of food, in part shortly before their transformation, while the others used far younger specimens. Similarly the experiments of Kellner and the following authors and also my own were made on young caterpillars while the observations of Krizenecky and of Szwajsówna refer to older larvæ. In another experimental study on starved tadpoles (Kopeć, 22*a* and *b*) I succeeded in confirming the above views of Wolterstorff, Morgan and others. My experiments showed that metamorphosis of the tadpoles to which starvation was applied before the fiftieth day of their development undergoes retardation, where as transformation is accelerated when tadpoles are starved after the sixty fifth day of their life. In this study on caterpillars similar behavior was also noted in one and the same material of female larvæ. It turned out that the caterpillars to which total starvation has been applied approximately since the seventh day after their last moult underwent pupation in three different broods after from 7 to 13, from 7 to 16 and from 7 to 15 days (or on the average after 10.6, 10.8 and 10.0 days), whereas the control specimens of these broods become chrysalids as early as after from 6 to 12, from 6 to 12 and from 5 to 12 days (or on the average after 8.2, 9.3 and 8.6 days). On the contrary the larval life of the females of the same broods in which starvation began approximately from the tenth day after the last moult was much shorter than that of control larvæ, as pupation occurred here from 4 to 8, from 5 to 8 and from 4 to 8 days (or on the average after 5.5, 6.6 and 6.8 days), in control specimens from 5 to 10, from 6 to 10 and from 7 to 9 days (or on the average after 7.4, 7.6 and 8.1 days). Hence follows: (1) The character of the influence, whether accelerating or retarding, exerted by starvation on the processes of metamorphosis depends on the period of life or the developmental stage on which the factor begins. (2) This moment may be accurately determined by means of experiment, viz, in the females of *Lymantria dispar* L. In my cultures the critical moment during which the influence of inanition delaying metamorphosis is changed into an accelerating one fell on the period between approximately the seventh and the tenth day after the last moult.

An essential explanation of such different behavior of caterpillars may be found in my former studies on the importance of the brain for insect metamorphosis (Kopeć, '17 and '22*c*). It was shown that the female caterpillars of this moth, deprived of brain the seventh day after their last moult, live far longer than control animals, but they die without undergoing transformation. On the other hand, the removal of the brain from caterpillars the tenth day after their last moult has no influence either on the processes of pupation or on the emergence of moths. From various experiments supported by observation on control material it follows that the brain plays here most probably the rôle of an organ of internal secretion. From these results we may infer that by depriving caterpillars of food since the tenth day after their last moult we afford exceptionally favorable conditions for metamorphosis, viz, the substance or substances already produced by the brain in sufficient quantities find in such starved organisms less material which ought to be transformed in time. The prolongation of the larval period in younger caterpillars starved before the seventh day after the last moult may be explained, on the contrary, by impeded secretoric function of the brain or by certain anomalies of its activity in starved organisms.

If the processes occurring in the pupa were a continuation of those taking place in the caterpillar and leading to pupation, the abbreviation of the pupal stage in starved specimens could not be reconciled with the above explanation. But these processes differ not only energetically as it has been shown by Tangl ('09*b*). The processes of pupation may morphologically be reduced to the histolysis of almost all larval tissues into a homogeneous mass which fills up the pupal body, whereas the transformation of the pupa into the fully-developed insect consists in the developemnt of the so-called imaginal discs, *i.e.*, of small accumulations of cells. In my present experiments (series *B*) I succeeded moreover in establishing certain physiological characters which point to another difference. In series *B* the caterpillars were deprived of food every second day for 20 days after their hatching from eggs, *i.e.*, at a time when there are not even any traces of histolytic processes in the larval body. From the twenty-first day till the end of the larval period the animals were fed every day and underwent the normal number of moults. From the appertaining items on Tables II. and III. we see that the caterpillars belonging to this series not only remarkably delayed the term of their pupation, but also that the duration of the pupal stage was considerably shortened. This abbreviation amounted in the average of broods to 17.4 per cent. of the average duration of life of normal pupae in males and to 22.5 per cent. in females. Consequently, the evolutive processes characteristic of the pupa are not a continuation of those changes which are characterized by histolysis of larval tissues. We see that by temporarily starving young caterpillars (Series *B*) we may segregate these processes and prove that processes of the development of the imaginal discs may begin far earlier, *i.e.*, soon after the hatching of the caterpillar from the egg. It ought therefore to be inferred that the brain has two separate functions in normal conditions, (1) it causes histolysis of larval tissues, (2) it delays the evolution of imaginal discs. During inanition of caterpillars the influence of the brain hindering the development of imaginal discs is decreased, owing to its lowered function. The starved chrysalids therefore begin their pupal stage when the discs are better developed and this causes acceleration of the development of the imaginal body and hence abbreviation of the pupal life.

The assumption that the larval brain exerts two different influences is neither astonishing nor incomprehensible in respect to the well-known data concerning the physiology of organs of internal secretion. The final development of the imaginal discs and their definitive differentiation sets in before pupation or even in the pupa, *i.e.*, at a time when almost all larval tissues are undergoing histolysis or have undergone it. Therefore it ought to be admitted that during this period the influence of the brain hindering the development of embryonal discs normally becomes annuled by certain processes which are unknown to us. It is very probable that the development of imaginal discs and the histolysis of the larval tissues become, at least towards the end of larval life, physiologically correlated with each other. In the contrary case it could be hardly understood why the caterpillars deprived of their brain the seventh day after their last moult do not, it is true, exhibit any histolytical changes in their tissues, but the imaginal discs contained in their body do not undergo final growth and differentiation. (Kopeć, '17 and '22 *c*). Indeed, if there were no such correlation, the imaginal discs of the caterpillars deprived in that period of their brain (and therefore of the organ retarding their evolution) ought to develop the organs of the imago in spite of the absence of histolytical processes in the larval body.

In contrast to my experiments on the starvation of caterpillars, Loeb and Northrop ('17) have lately convinced themselves that each of the separate stages of life in Drosophila occurs more slowly in lower temperature and more quickly in higher ones. The changes of temperature were applied by the mentioned authors during the whole development of the animals, while in my experiments the factor of inanition had a direct influence only on the processes taking place in caterpillars, as the chrysalis does not take food in normal conditions either. Loeb and Northrop obtained the same changes of the duration of the larval and of the pupal stage first of all owing to changed celerity of metabolism in the larva as well as in the pupa. Experiments in which lower or higher temperatures should be applied only to larvae, the chrysalids being kept in normal conditions, might solve the problem as to whether and to what degree the changed temperature has an influence not only on the celerity of metabolism, but also on the supposed function of the brain.

The attempts hitherto made to explain the metamorphosis of insects refer to the last stage of the processes. The appearance of phagocytosis, of degeneration, of asphyxia and of other "causes" of transformation is not yet elucidated. My experiments on the function of the larval brain as well as the discovery of tyrosinase in caterpillars and chrysalids made by Dewitz ('05 and '16) and confirmed by Steche and Waentig ('13) together with the present results on the starvation of caterpillars may lead to a better knowledge of the cause of insect metamorphosis. But my "secretory" theory of metamorphosis will not be well grounded until we succeed in finding in the structure of the brain an adequate base for the theory, *i.e.*, until certain specific changes in the brain not only during pupation, but also during moults will have been ascertained. A great support might be afforded by positive experimental results on transplantation of brains, or on injections of extracts of this organ into brainless specimens. The latter experiments only would be able to dispel every doubt in regard to the validity of my previous conclusion that the indubitable influence of the brain on metamorphosis is due to an internal secretion of this organ.

Deegener ('09) lastly considers the imaginal form to be phylogenetically older than the larval form, which developed secondarily from the fully developed insect owing to numerous secondary life conditions "unter fortgehender Retardation der Entwicklung imaginaler Organe" (p. 11). I think that my experiments of series *B* distinctly prove that in normal circumstances the development of the imaginal discs is retarded during larval stages. I cannot deny that in the light of these results the supposed retardation of the development of the imaginal organs gains an experimental base, at least in ontogenetic evolution.

3. Inanition and Certain Problems of Growth.

In this chapter I take into account only the final stages of growth of caterpillars expressed by the weight of new-formed pupæ. The chrysalids were weighed from 1 to 24 hours after pupation, control weighings having proved that the weight of pupæ decreased in my broods during this period only from 0.18 per cent. to 0.33 per cent. The limits of fluctuations in the weight of pupæ are recorded in Table I., together with their average values. On Table III. we see moreover the average weight of "starved" chrysalids of each brood calculated in percentages of the average weight of control chrysalids of the same sex and brood.

On comparing the data of series *C*, *D* and *E* we see that the weight of chrysalids decreases more and more in proportion as the number of fasting days increases. Assuming in general that the number of feeding days in separate series corresponds to the quantity of food which has been taken, we may say that the weight of pupæ is in direct relation to the quantity of food consumed. If we also take into consideration the data of series *F* in comparison to analogous items of series *D*, we draw the conclusion that the average decrease of chrysalids due to inanition of caterpillars is larger when the feeding intervals are longer though less frequent. As it was pointed out, the prolongation of the stage of caterpillar and the abbreviation of that of pupa increases in proportion as more and more intense starvation has been applied. Consequently, the weight of the pupæ the caterpillars of which had been starved is in inverse relation to the prolongation of the larval period as well as to the abbreviation of the stage of chrysalids. Adopting the ratio of the pupal weight to the duration of larval life as the approximative measure of the rate of growth, we can infer from Tables I. to III. that this rate decreases in proportion as more and more intense starvation has been applied and in relation to the distribution of the feeding and fasting days (Series *F* and *D*). The above-stated principles refer to separate series of experiments but not to separate specimens, either control or starved, in one brood. I have often observed that although the processes of transformation lasted in the control or in the starved material in every brood several days, the heaviest and the lightest caterpillars underwent pupation the same day, sometimes the first and sometimes not until the last day, although the one was two- or threefold heavier than the other. The same may be said as to the duration of pupal life in regard to the weight of separate chrysalids of one brood.

The problem arises whether the capacity to grow is checked by age of the animal. Such limits in rats have been adopted by Aron ('12) and lately by Jackson and Stewart ('20), in contrast to Osborne and Mendel ('14) who are inclined to the opinion that the capacity to grow is exhausted by mere growth, without regard to the factor of time. In series *B* of my experiments the caterpillars, deprived of food intermittently for 20 days since their hatching, weighed the twenty-first day in separate broods on the average only 7.6, 3.6 and 5.0 mg., while control individuals of the same broods had the mean weight of as much as 36.9, 45.5 and 40.3 mg. The starved specimens fed daily since the twenty-first day attained and partly exceeded in time the weight and size of control caterpillars (cf. weights on Table I. and III.). As in this series the processes of metamorphosis of originally starved caterpillars were retarded and as, on the other hand, caterpillars have grown until the end of their larval life, it ought to be inferred that in these animals the capacity to grow is not suppressed at a period at which caterpillars normally cease to grow. Moreover, it may be concluded that pupation also may take place much beyond the age at which the control specimens undergo pupation. But the inference that the capacity may not at all be limited by the age of animals ought not to be drawn from such results. It is possible that the rats experimented upon by Mendel and Osborne as well as my caterpillars would lose the capacity to obtain the weight and size of control specimens if they had been kept at maintenance or starved even a few days longer.

4. Adaptation of Organisms to Starvation. Some Remarks on the Problem of Death.

The weight of pupae from series *A* (in which starvation lasting one day every second day, + − + − + −, was applied to caterpillars during the whole larval life) is evidently different from that of series *D* in which older specimens after their last moult but one were subjected to the same starvation (cf. Table I.). We see that organisms may in time get accustomed to the detrimental effects of starvation, which prevent the animal from attaining its normal weight: the comparison of the limits of individual fluctuations shown on Table I. points to the conclusion that the chrysalids of series *D* which have been deprived of food only during the half of their larval life are lighter than those from series *A* the caterpillars of which have been starved during their whole larval life. From Table III. we see that the male pupæ from series *D* weighed in the average of broods 48.5 per cent., the female pupæ 48.4 per cent. of the average weight of the control chrysalids, whilst the male chrysalids from series *A* weighed as much as 70.2 per cent., the females 78.3 per cent.

As the prolongation of the larval period was much greater in series *A* than in series *D* (cf. Table I.), it might be supposed that the caterpillars of series *A* are heavier owing to the circumstance that they lived longer, and therefore could take and digest food during a longer period. But the following argument contradicts this opinion. At the outset of the experiment in series *D* the caterpillars weighed in the average of broods 94.5 mg., the male specimens underwent pupation in the average of broods after 26.1, the female after 29.7 days. If we take as starting point for series *A* the day on which the caterpillars of this series had an analogous weight, which in the average of broods amounted to 93.5 mg., the duration of larval life in this series from this day till pupation was in the average of broods 27.6 in males and 36.2 in females. We see that when the starting point had been made uniform the duration of farther larval life in series *A*, especially in males, is almost identical with that in series *D*. In other words, the specimens of series *A* attain considerably greater weight than those of series *D* during approximately the same period. The ratio of the produced number of milligrams of body-weight to the total number of days of the period during which the larvæ had been deprived of food being considered as rate of growth, we may calculate that the rate of growth amounts in the average of broods in series *A* to 7.4 in starved males and to 19.8 in females, whilst in series *D* this quantity attains only 4.5 in starved males and 15.8 in females. It follows that the starved organism may in time get accustomed to the metabolism of inanition, *i.e.*, the ratio of assimilation to disassimilation becomes during long starvation changed in favor of the organism.

By histological research I have convinced myself that caterpillars which died from starvation contain no adipose tissue. In a certain contrast to my observations, Bialaszewicz ('19) draws from his experiments on the starvation of leeches as well as from the papers by several authors on other animals the conclusion supported by numerous grounds that fat has no great importance in the hunger metabolism of cold-blooded animals, but that it undergoes only small reduction. The farther investigations of Bialaszewicz will undoubtedly show whether my supposition based on histological research is right, or they may explain the cause of this discrepancy which possibly consists in the physiological capacity of caterpillars to digest their store of adipose tissue during their frequent moults.

In contemporary research the cause of natural death is regarded as caused by the accumulation of detrimental products of normal metabolism which cannot be removed from the multicellular organism. This view is not at all proved; it is based first of all on the known investigations of Woodruff on the infusoria whose conclusions are in discrepancy with the results obtained by Viewegerowa and Vieweger ('22) who by methodically exact research proved that the products of metabolism have no great importance on the development of Colpidium and that the divisions are hindered in unchanged surroundings first of all by inanition. It nevertheless seems to me to be unquestionable that the duration of life depends on the character of metabolism, in other words, that natural death is a function of metabolism. The experiments performed by Kellogg and Bell ('04 *b*), by Pictet ('04) and by Northrop ('17) on larvæ as well as those by Loeb and Northrop ('17) on fully-developed insects of Drosophila show that by means of inadequate food we may elicit considerable changes of the duration of development or of life of these organisms. As investigations on the hunger metabolism evidence distinct differences of the digested substances and of the character of their disintegration in comparison to normal metabolism, it ought to be inferred that, if death is a function of metabolism, insufficient feeding may also influence the moment of natural death. Schultz ('05) emphasizes that certain animals undergoing periods of hibernal (or æstival) sleep which is connected with very restricted metabolism live very long in comparison with those having no such periods of rest. Stress must be laid on the fact that in my experiments moths deriving from starved caterpillars in which the development was much prolonged or delayed are much older organisms than the control moths. As the imago of *Lymantria dispar* L. never takes any food, the quantity of provisions stored in its body decides the duration of this life-period. From histological research it follows that the amount of this provision which may be noticed in the imago (the so-called adipose body) is in the starved specimens, in relation to their decreased body, not at all smaller than in the controls. This is due to the fact that the starved caterpillars do not form normally sized chrysalids, which contain smaller quantities of the mentioned body, but they are transformed into smaller, even dwarfed specimens. By this circumstance it might be explained why both categories of moths live in general equally long. But in the case of the "starved" moths derived from caterpillars the life of which has undergone a very remarkable prolongation it is obvious that the hunger metabolism during development caused retardation of natural death of the organism. This I consider to point clearly to the conception of death as a function of the character of general metabolism. According to Ruzicka's researches ('17), the newts, when totally deprived of food, undergo moults more rapidly than control specimens. In connection with my experiments it would be very interesting to ascertain whether the duration of life of such newts undergoes changes.

More detailed considerations on the cause of especially favorable influence of intermittent starvation on duration of life of animals are to be found in my former papers (Kopeć, '22 *a* and *b*.)

5. Summary.

1. Intermittent starvation of young caterpillars of *Lymantria dispar* L. causes considerable prolongation of the larval life as well as a certain abbreviation of the pupal period but has no influence on the duration of life of the imago. These changes increase in proportion as more intense starvation is applied. Larger effects are elicited by longer and less frequent than by more frequent, but shorter, feeding intervals.

2. The differences of results obtained by various authors in regard to the influence of starvation on metamorphosis depend on differences of age of the animals experimented upon. The caterpillars subjected to inanition approximately from the seventh

day after their last moult had retarded pupation, whereas this process is accelerated by starvation of animals approximately since the tenth day after the last moult.

3. The development of imaginal discs is not the consequence of histolytical processes which cause pupation of caterpillars, but they take place simultaneously from the first days of larval life. The brain causes, probably by its secretion (or secretions), histolysis of larval tissues and it also seems to check in the caterpillar the development of the imaginal discs.

4. The prolongation of the larval period in starved specimens may be explained by certain disturbances in the hypothetical secretory function of the larval brain, which are caused by inanition; the abbreviation of the pupal stage may be ascribed to analogous decrease of the influence of this organ, which retards the development of imaginal discs.

5. The average limit of larval growth expressed in the average weight of the new-formed chrysalids is in direct proportion to the quantity of food given and inverse to the prolongation of larval and to the abbreviation of pupal life. (The decrease of weight of caterpillars is larger in cases of longer though more rare food-intervals than in cases of more frequent but shorter ones.) These rules may be applied only to differently starved whole experimental materials, having no application to separate specimens of separate broods, both starved and control.

6. The capacity to grow as well as the capacity to undergo metamorphosis exists in starved specimens far beyond the age at which control caterpillars cease to grow and undergo transformation.

7. During long lasting starvation organisms get accustomed to the abnormal conditions: the rate of growth of the caterpillars starved every second day during their whole life becomes in time considerably greater than that of specimens analogically deprived of food since their last moult but one.

8. Hunger death of caterpillars is probably caused first of all by exhaustion of reserve substances. Natural death of the imago probably is a function of the character of metabolism, as death is delayed by the changed metabolism of intermittent starvation.

BIBLIOGRAPHY.

Papers marked by an asterisk are known to me only from abstracts.

* **Aron, H.**
'12 Weitere Untersuchungen über die Beeinflussung des Wachstums durch die Ernährung. Verh. d. Gesell. f. Kinderheilkunde, Münster.

Barfurth, D.
'87 a Versuche über die Verwandlung der Froschlarven. Arch. f. mikr. Anat., Vol. 29, p. 1.
'87 b Der Hunger als förderndes Prinzip in der Natur. Ibidem, p. 28.

Bialaszewicz, K.
'19 Etudes comparées sur le métabolism chimique et énergetique. 1. L'inanition et la nutrition chez les Hirudinees. Trav. de la Soc. des Sc. de Varsovie, III., Nr. 32.

Deegener, P.
'09 Die Metamorphose der Insecten. Teubner, Leipsic and Berlin.

Dewitz, J.
* '05 Untersuchungen über die Verwandlung der Insectenlarven. II. Arch. f. Anat. und Physiol., Physiol. Abt., Suppl.
'06 III. Zusammenfassung früherer Mitteilungen. Zool. Anz., Vol. 47.

* **Jackson, C. M., and Stewart, C. A.**
'20 The Effects of Inanition in the Young upon the Ultimate Size of the Body and of the Various Organs in the Albino Rat. Journ. of Exper. Zoöl., Vol. 30.

Kaufman, L.
'18 Researches on the Artificial Metamorphosis of Axolotls. Bull. Ac. Sc. Cracovie.

Kellner, O.
'87 Chemische Untersuchungen über die Ernährung und Entwicklung des Seidenspinners (*Bombyx mori*). Landw. Versuchst., Vol. 33.

Kellogg, V. L., and Bell, R. G.
'04 a Notes on Insect Bionomics. Journ. of Exper. Zoöl., Vol. 1.
'04 b Variations Induced in Larval, Pupal and Imaginal Stages of *Bombyx mori* by Controlled Varying Food Supply. Science, N. S., Vol. 18 (Leland Stanford Junior University Publications, University Series, No. 1).

Kopeć, S.
'17 Experiments on Metamorphosis of Insects. Bull. Ac. Sc. Cracovie.
'22 a Further Research on the Influence of Inanition on the Development of Animals. Experiments on Tadpoles. Mem. de l'Institut Nation Polonais d'Économie Rurale à Pulawy, Vol. 3.
'22 b Experimental Studies on the Influence of Inanition on the Development and the Weight of Amphibians. Bull. Ac. Pol. Sc., Cracovie.
'22 c Studies on the Necessity of the Brain for the Inception of Insects' Metamorphosis. BIOL. BULL., Vol. 42.

Krizenecky, J.
'14 Über die beschleunigende Einwirkung des Hungers auf die Metamorphose. Biol. Centrbl., Vol. 34.

Loeb, J., and Northrop, J. H.
'17 On the Influence of Food and Temperature upon the Duration of Life. Journ. of Biol. Chem., Vol. 32.

Morgan, Th. H.

'07 Experimental Zoölogy, New York.

* **Northrop, J. H.**

'17 The Effect of Prolongation of the Period of Growth on the Total Duration of Life. Journ. of Biol. Chem., Vol. 32.

* **Osborne, T. B., and Mendel, L. B.**

'14 The Suppression of Growth and the Capacity to Grow. Journ. of Biol. Chem., Vol. 18.

Pictet, A.

'05 Influence de l'alimentation et de l'humidité sur la variation des papillions. Mém. de la soc. de physiques et d'hist. nat. de Genève, Vol. 35.

Ruzicka, V.

'07 Beschleunigung der Hautung durch Hunger. Arch. f. Entw. Mech., Vol. 42.

Schultz, E.

'05 Über Verjungung. Biol. Centrbl., Vol. 25

* **Steche, O. u. Waentig, P.**

'13 Untersuchungen über die biologische Bedeutung und Kinetik der Katalase. Zoologica, Vol. 26.

Szwajsówna, P.

'16 Le métabolisme physiologique chez les larves du Tenebrio molitor. Comptes rend. Soc. Sc. de Varsovie, Vol. 9.

Tangl, F.

'09 a Zur Kenntnis des Stoff- und Energieumsatzes holometaboler Insekten wahrend der Metamorphose. Arch. f. d. ges. Physiol., Vol. 130, p. 1.

'09 b Embryonale Entwicklung und Metamorphose vom energetischen Standpunkte aus betrachtet. Ibidem, p. 55.

Viewegerowa, J., and Vieweger, T.

'21 Récherches sur les causes du developpement des cultures du Colpidium colpoda Travaux du labor physiol. de l'Inst. M. Nencki, Varsovie, Vol. 1.

Wolterstorff, W.

'96 Über die Neotenie der Batrachier. Zool. Garten, Vol. 37.